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Withdrawal From Continuous or Intermittent Cocaine: Effects of NAN-190 on Cocaine-Induced Locomotion

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KING, G. R., C. JOYNER, T. H. LEE AND E. H. ELLINWOOD, JR. *Withdrawal from continuous or intermittent cocaine: Effects of NAN-190 on cocaine-induced locomotion.* PHARMACOL BIOCHEM BEHAV **44**(2) 253–262, 1993. – Rats were pretreated with 40 mg/kg/day cocaine for 14 days by either SC injections or osmotic minipumps. Rats were then withdrawn from the pretreatment regimen for 7 days. In Experiment 1, rats received 0- to 2.0-mg/kg IP injections of 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190), a putative 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor antagonist. In Experiment 2, rats received the same doses of NAN-190 in combination with a 15-mg/kg IP injection of cocaine. The results of Experiment 1 indicate that the continuous-infusion group demonstrated a dose-dependent suppression of locomotor behavior by single doses of NAN-190. NAN-190 had no consistent dose-dependent effect on the locomotor behavior of subjects in the other pretreatment groups. The results of Experiment 2 indicate that rats receiving intermittent, daily injections tended to exhibit behavior consistent with 5-HT$_{1A}$ receptor supersensitivity. In contrast, rats receiving continuous cocaine tended to exhibit behavior consistent with 5-HT$_{1A}$ receptor subsensitivity. Changes in 5-HT$_{1A}$ receptor sensitivity may contribute to some of the anxiety and depressive symptoms exhibited by human cocaine abusers.

Cocaine withdrawal 5-HT$_{1A}$ receptors Sensitization Tolerance Rats

RESEARCH on chronic cocaine administration indicates that both the dose and route of administration influences the effects of chronic cocaine [see (19) for a review]. For example, daily intermittent IP injections of cocaine produce sensitization (i.e., reverse tolerance) to the locomotor- and stereotypy-inducing properties of cocaine (22,27,31). Schedule-induced cocaine intake has also been found to produce sensitization; however, oral administration of a single, daily dose of cocaine does not (11,22). Lastly, daily SC injections also produce sensitization to the locomotor effects of cocaine, while continuous infusion of cocaine via the Alza (Alza Corp., Palo Alto, CA) minipump produces tolerance (21,29). These results indicate that the behavioral effects of chronic cocaine are partially dependent upon the method and temporal pattern of administration.

The exact mechanisms underlying these behavioral effects of chronic cocaine administration are unknown. However, it is in general thought that the psychomotor stimulant and stereotypy-inducing properties of cocaine are partially mediated by mesolimbic and nigrostriatal dopamine release [DA; see (19) for a review]. Although this research indicates that the behavioral effects of cocaine are correlated with DA neurotransmission, several lines of evidence indicate that the serotonin [5-hydroxytryptamine (5-HT)] system has an inhibitory modulating role in stimulant-induced behavior. For example, midbrain serotonergic raphe (5,18,24) or medial forebrain bundle lesions (13) enhance the locomotor-stimulating effects of amphetamine. Also, administration of parachlorophenylalanine or 5,6- or 5,7-dihydroxytryptamine also enhances the locomotor-stimulating properties of the amphetamines (3,23, 25,32). Hence, this research seems to indicate that there is an inverse relation between 5-HT neurotransmission and the locomotor-activating properties of psychomotor stimulants.

Central 5-HT receptors have been divided into four general classes: the 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ receptors. The 5-HT$_1$ class demonstrates extensive heterogeneity and has been further divided into four subtypes: the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$ receptors (26). The 5-HT$_{1A}$ receptor is extensively distributed in the limbic areas such as the hippocampus, lateral septum, the frontal and entorhinal cortex, the central amygdala, and the dorsal and median raphe. This receptor is not, however, extensively found in the hypothalamus, thalamus, and extrapyramidal areas such as the caudate putamen, globus pallidus, and substantia nigra (15). Lesion studies indicate that dorsal raphe 5-HT$_{1A}$ receptors are located presynaptically (35), while hippocampal 5-HT$_{1A}$ receptors are located postsynaptically (14).

Activation of the 5-HT$_{1A}$ receptor results in a behavioral

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pattern consisting of hyperlocomotion, head-weaving, flat body posture, and reciprocal forepaw treading (1,2,17,34). Some of the behavioral effects of 5-HT_{1A} receptor stimulation are blocked by the putative 5-HT_{1A} antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190) (12, 31). A role for catecholamines in the hyperlocomotion produced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} agonist, has been demonstrated by Tricklebank et al. (34). Also, cocaine has been shown to decrease the firing rates of dorsal raphe neurons (7,8) via activation of presynaptic 5-HT_{1A} autoreceptors.

Cocaine is a 5-HT uptake inhibitor (30) and has substantial effects on serotonergic neuron electrophysiology (6–9). Further, Broderick (4) found that SC injections of cocaine resulted in a dose-dependent decrease in synaptic 5-HT levels in the nucleus accumbens. Given these effects of cocaine on 5-HT neuronal functional, it is distinctly possible that some of the behavioral effects of chronic cocaine administration (e.g., sensitization and tolerance) may be partially mediated by changes in 5-HT receptor sensitivity.

The present experiments examine changes in 5-HT_{1A} receptor sensitivity produced by withdrawal from different patterns of cocaine administration (i.e., continuous or intermittent cocaine). Rats were pretreated for 14 days with either continuous or intermittent daily injections of cocaine. Subjects were then withdrawn from the pretreatment regimen for 7 days and changes in 5-HT_{1A} receptor sensitivity were assessed by behavior ratings over several challenge doses of the 5-HT_{1A} receptor antagonist NAN-190 alone (Experiment 1) and in combination with a 15-mg/kg IP dose of cocaine (Experiment 2).

METHOD

Animals

Male Sprague-Dawley rats, initially weighing 100–125 grams (Charles River Laboratories), were acclimated to the vivarium on a 12 L : 12 D (light between 7:00 a.m.–7:00 p.m.) for 1 week prior to treatment. They were housed in pairs in plastic cages with continuous access to food and water.

Drugs

Cocaine HCl (received from NIDA) was dissolved in 0.9% sterile saline. NAN-190 HBr (obtained from Research Biochemicals, Inc.) was dissolved in DMSO. All doses are calculated as the base, and injection volume was based upon the body weight.

Minipump Preparation

Alzet Osmotic Pumps (Model 2ML2) from Alza Corp. were filled with either 2 ml 100 mg/ml cocaine HCl or saline (0.9%); the infusion rate was 2 μl/h, resulting in an overall average dose of 40 mg/kg/day for the cocaine pumps. The pump was primed by warming in a beaker of saline in a waterbath at 37°C for 4 h prior to surgical implantation.

Surgery

Animals were shaved and injected locally with (0.2 cc) lidocaine (Abbott, North Chicago, IL) at the dorsal midline incision site. Animals were then anesthetized by inhalation with methoxyflurane (Metofane). A 2-cm vertical incision was made with scissors and a large SC pocket was formed with the scissors. The minipump was inserted into this pocket with the delivery portal toward the head. The opening was closed with metal surgical autoclips. On day 14, the pumps were surgically removed using the same procedure and the residual amount of cocaine measured. The amount was consistently less than 15% of the original volume, indicating that rats approximately received the programmed daily dose.

Pretreatment

Pretreatment was for a 14-day period. On day 1 of treatment, animals were either: a) implanted with 2ML2 Alzet minipumps continuously infusing cocaine at a rate of 40 mg/kg/day (continuous infusion group), b) injected SC once daily with 40 mg/kg cocaine HCl (injection group), or c) injected SC with 0.9% saline (saline control group) once daily. There was no saline pump control group as the results of King et al. (21) indicated that there were no differences in the behavioral responses to cocaine challenges in rats that had received either saline injections or saline pumps. Thus, the surgery itself had no effect on the behavioral response to cocaine.

Behavioral Testing

On day 7 following pretreatment, animals were acclimated to the test room in their home cage for 30 min under normal light conditions. The test cages were standard, clear plastic laboratory animal housing cages, 28 × 18 × 12 cm, with another cage taped, upside down, in place on top. The top cage had five air holes drilled uniformly on either side. Six of these test cages were placed in a row 12 in. apart. A modified version of the Ellinwood and Balster Rating Scale (10) was used (Table 1). A rating was given to each of the animals at 5-min preinjection and at 5-min intervals thereafter, for a total of 60 min. The observation period was for 20 s with 10 s between cages.

For the test session in Experiment 1, each rat received one of the following doses of NAN-190 IP: 0, 0.5, 1.0, or 2.0 mg/kg 30 min prior to the session. For the test session in Experiment 2, each rat received one of the following doses of NAN-190 IP—0, 0.5, 1.0, or 2.0 mg/kg 30 min prior to the session—and a 15-mg/kg IP cocaine injection immediately prior to the session.

For each test session in both experiments, the subject types (i.e., injection, pump, saline) were randomized according to a Latin square design; the doses for each test session were also randomized by a Latin square design. The significance level was set at $p < 0.05$ for all comparisons. There were 10 rats per condition.

RESULTS

Experiment 1

Figure 1 presents the mean behavior rating for each dose of NAN-190 separately for each pretreatment group. Panel A presents the behavior ratings of the saline control group. Kruskall–Wallis analyses of variance (ANOVAs) by ranks were performed separately at each time point to determine if there were any differences in the behavior ratings across the doses of NAN-190. The results of these tests indicated that there was a significant dose effect only at 25 min. Mann–Whitney *U*-tests comparing the different doses of NAN-190 at 25 min indicated that the 0.5-, 1.0-, and 2.0-mg/kg doses all resulted in ratings significantly smaller than the 0.0-mg/kg dose.

Similar to panel A, panel B presents the behavior ratings for the cocaine injection groups separately for each dose of

TABLE 1

MODIFIED ELLINWOOD AND BALSTER (10) RATING SCALE

Score		Definition
1	Asleep	Lying down, eyes closed
2	Almost asleep	Relaxed muscles, eyes partially shut
3	Dystonia	Abnormal posture, tense muscles
4	Inactive	Lying down, eyes open, infrequent sniffing
5	In-place oral behavior	Vacuous oral movements, jaw tremor, yawning
6	Grooming	Grooming of face, body, or groin
7	Normal active movement	Investigation of sniffing of cage, rearing
8	Hyperactive	Running movement characterized by rapid changes in position (jerky)
9	Slow patterned movement	Repetitive exploration of the cage at normal levels of activity
10	Fast patterned movement	Repetitive exploration of the cage with rapid, intense, stereotyped activities
11	Stereotypy	Types of stereotypies are noted
12	Hyperreactive	The following types of behavior are described and/or counted: jerky hyperactive movements, jumping (popcorn)-like movements, seizures, disjunctive, movements, obstinate regression (backing up)

NAN-190. The results of Kruskall-Wallis ANOVAs by ranks did not indicate any significant differences at any time point.

Similar to panels A and B, panel C presents the behavior ratings for the continuous infusion group separately for each dose of NAN-190. The results of Kruskall-Wallis ANOVAs by ranks indicated that there was a significant dose effect at 5 min and 20–30 min. Mann-Whitney *U*-tests comparing the different doses of NAN-190 at 5 min indicated that the 1.0-mg/kg dose resulted in behavior ratings significantly smaller than the 0.0-mg/kg dose. Mann-Whitney *U*-tests comparing the different doses of NAN-190 at 20 min indicated that the 1.0- and 2.0-mg/kg doses resulted in behavior ratings significantly smaller than the 0.0-mg/kg dose. Mann-Whitney *U*-tests comparing the different doses of NAN-190 at 25 min indicated that the 1.0- and 2.0-mg/kg doses resulted in behavior ratings significantly smaller than the 0.0-mg/kg dose. Mann-Whitney *U*-tests comparing the different doses of NAN-190 at 30 min indicated that the 1.0- and 2.0-mg/kg doses resulted in behavior ratings significantly smaller than the 0.0-mg/kg dose. No other comparisons were significant.

Figure 2 presents the mean behavior rating for each pretreatment group separately for each dose of NAN-190. Panel A presents the behavior ratings of subjects receiving a vehicle injection. The results of Kruskall-Wallis ANOVAs by ranks did not indicate any significant differences at any time point.

Similar to panel A, panel B presents the behavior ratings for subjects receiving a 0.5-mg/kg NAN-190 challenge dose. The results of Kruskall-Wallis ANOVAs by ranks did not indicate any significant differences at any time point.

Similar to panels A and B, panel C presents the behavior ratings for subjects receiving a 1.0-mg/kg NAN-190 challenge dose. The results of Kruskall-Wallis ANOVAs by ranks indicated significant differences between the pretreatment groups at 5 and 30 min. Mann-Whitney *U*-tests comparing the different pretreatment groups indicated that the behavior ratings for the continuous-infusion group are significantly smaller than the behavior ratings for the injection group at both 5 and 30 min.

Similar to panels A, B, and C, panel D presents the behavior ratings for subjects receiving a 2.0-mg/kg NAN-190 challenge dose. The results of Kruskall-Wallis ANOVAs by ranks indicated significant differences between the pretreatment groups at 10, 20, and 25 min. Mann-Whitney *U*-tests comparing the different pretreatment groups indicated that the behavior ratings for the continuous-infusion group were significantly smaller than the behavior ratings for the injection group at 10, 20, and 25 min. Further, the behavior ratings for the saline control group were significantly smaller than those for the injection group at 20 min.

Experiment 2

Figure 3 presents the mean behavior rating for each combination of cocaine and NAN-190 separately for each pretreatment group. Panel A presents the behavior ratings of saline control group. In other words, panel A presents the dose-response curve for the saline group over the different NAN-190 plus cocaine combinations. Kruskall-Wallis ANOVAs by ranks were performed separately at each time point to determine if there were any differences in the behavior ratings for the different combinations of NAN-190 and cocaine. The results indicated that there was a significant dose effect at 15–45 and 55 min. Mann-Whitney *U*-tests comparing the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection with the 0.5-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that there were significant differences at 15, 25, and 30 min. Mann-Whitney *U*-tests comparing the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection with the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that there were significant differences at 15 and 55 min. Mann-Whitney *U*-tests comparing the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection with the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that there were significant differences at 15–45 and 55 min. Mann-Whitney *U*-tests comparing the 0.5-mg/kg NAN-190 plus 15-mg/kg cocaine injection with the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated no significant differences at any time point. Mann-Whitney *U*-tests comparing the 0.5-mg/kg NAN-190 plus 15-

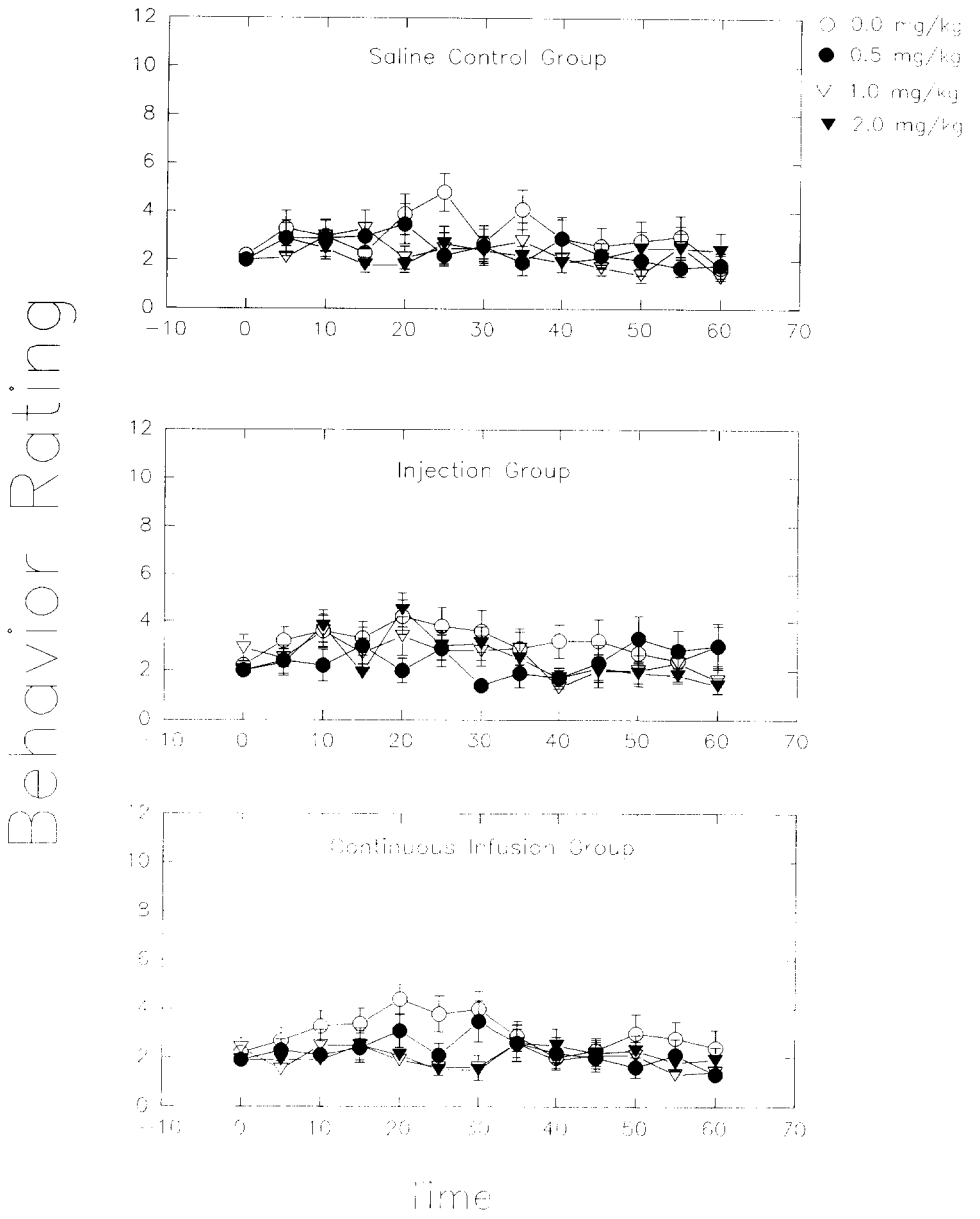

FIG. 1. Mean behavior rating for each dose of NAN-190 separately for each pretreatment group. The bars represent 1 SE. (○), vehicle (0.0 mg/kg) dose; (●), 0.5-mg/kg dose; (△), 1.0-mg/kg dose; (▲), the 2.0-mg/kg dose.

mg/kg cocaine injection with the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that subjects in the 0.5-mg/kg NAN-190 plus 15-mg/kg cocaine condition exhibited significantly higher behavior ratings than subjects in the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine condition at 20 and 35–45 min. Mann–Whitney *U*-tests comparing the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection with the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that subjects in the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine condition exhibited significantly higher behavior ratings than subjects in the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine condition at 20–45 min.

Similar to panel A, panel B presents the behavior ratings for the cocaine injection group. In other words, panel B presents the dose–response curve for the cocaine injection group over the different NAN-190 plus cocaine combinations. The results of Kruskall–Wallis ANOVAs by rank indicated that there were significant dose effects at 5–50 min. Mann–Whitney *U*-tests comparing the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection with the 0.5-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that there were significant differences at 10 and 20–40 min. Mann–Whitney *U*-tests comparing the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection with the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that there were significant differences at 5–50 min. Mann–Whitney *U*-tests comparing the 0.0-mg/kg

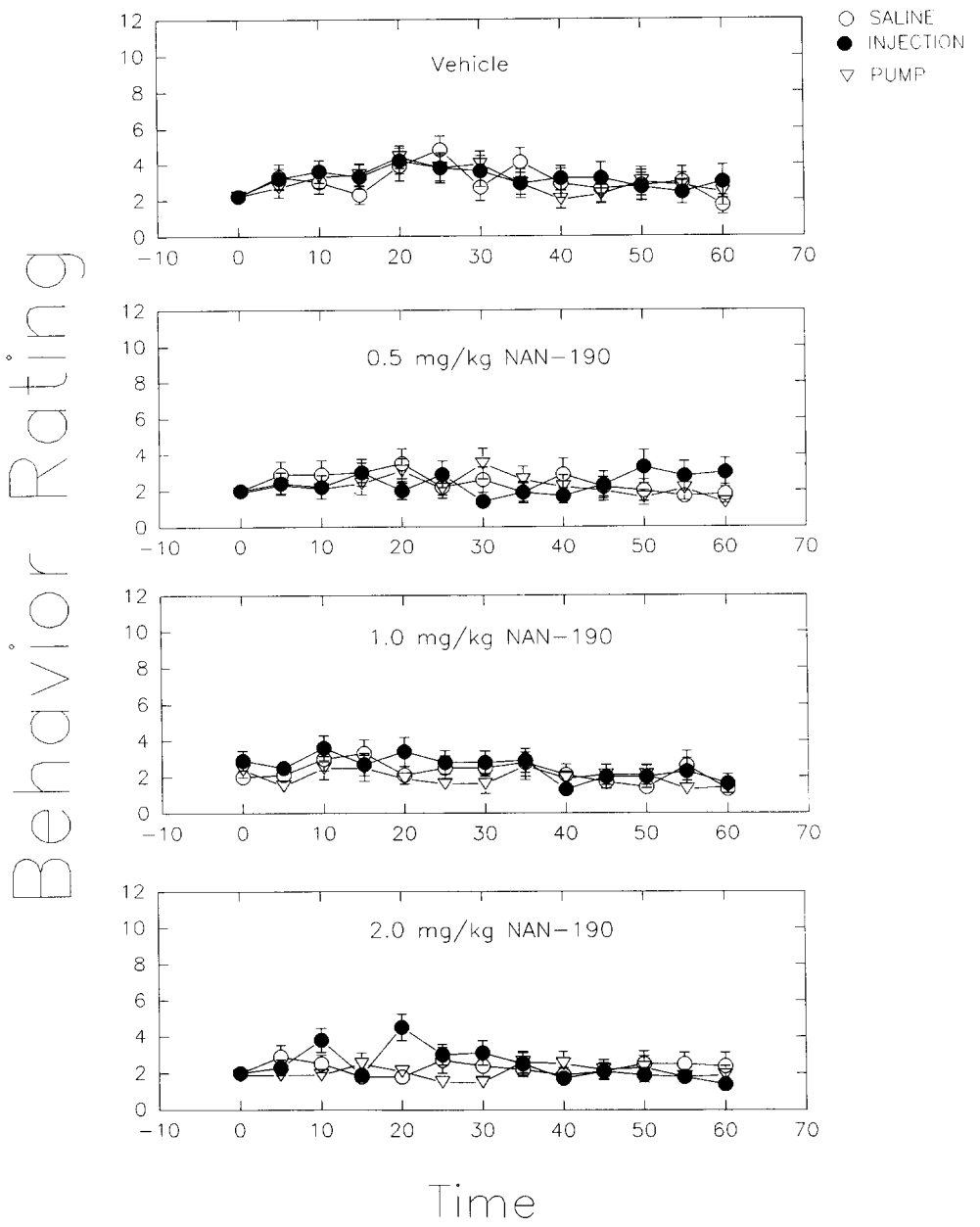

FIG. 2. Mean behavior rating for each group separately for each dose of NAN-190. The bars represent 1 SE. (○), saline pretreatment rats; (●), cocaine injection pretreatment rats; (△), continuous-infusion pretreatment rats.

NAN-190 plus 15-mg/kg cocaine injection with the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine injection indicated that there were significant differences at 10, 20–30, and 40–50 min. No other comparisons are significant.

Similar to panels A and B, panel C presents the behavior ratings for the continuous-infusion group. In other words, panel C presents the dose-response curve for the continuous-infusion group over the different NAN-190 plus cocaine combinations. The results of Kruskall-Wallis ANOVAs by rank indicated that there was a significant effect only at 35 min. Mann-Whitney *U*-tests indicated that the 0.5-mg/kg NAN-190 plus 15-mg/kg cocaine dose resulted in significantly higher behavior ratings than the 15-mg/kg cocaine dose, the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine dose, and the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine dose. No other comparison was significant.

Figure 4 presents the mean behavior rating for each pretreatment group separately for each combination of NAN-190 and 15 mg/kg cocaine. Panel A presents the behavior ratings of subjects receiving 0.0 mg/kg NAN-190 plus 15 mg/kg cocaine. The results of Kruskall-Wallis ANOVAs by rank indicated that there were significant differences between the

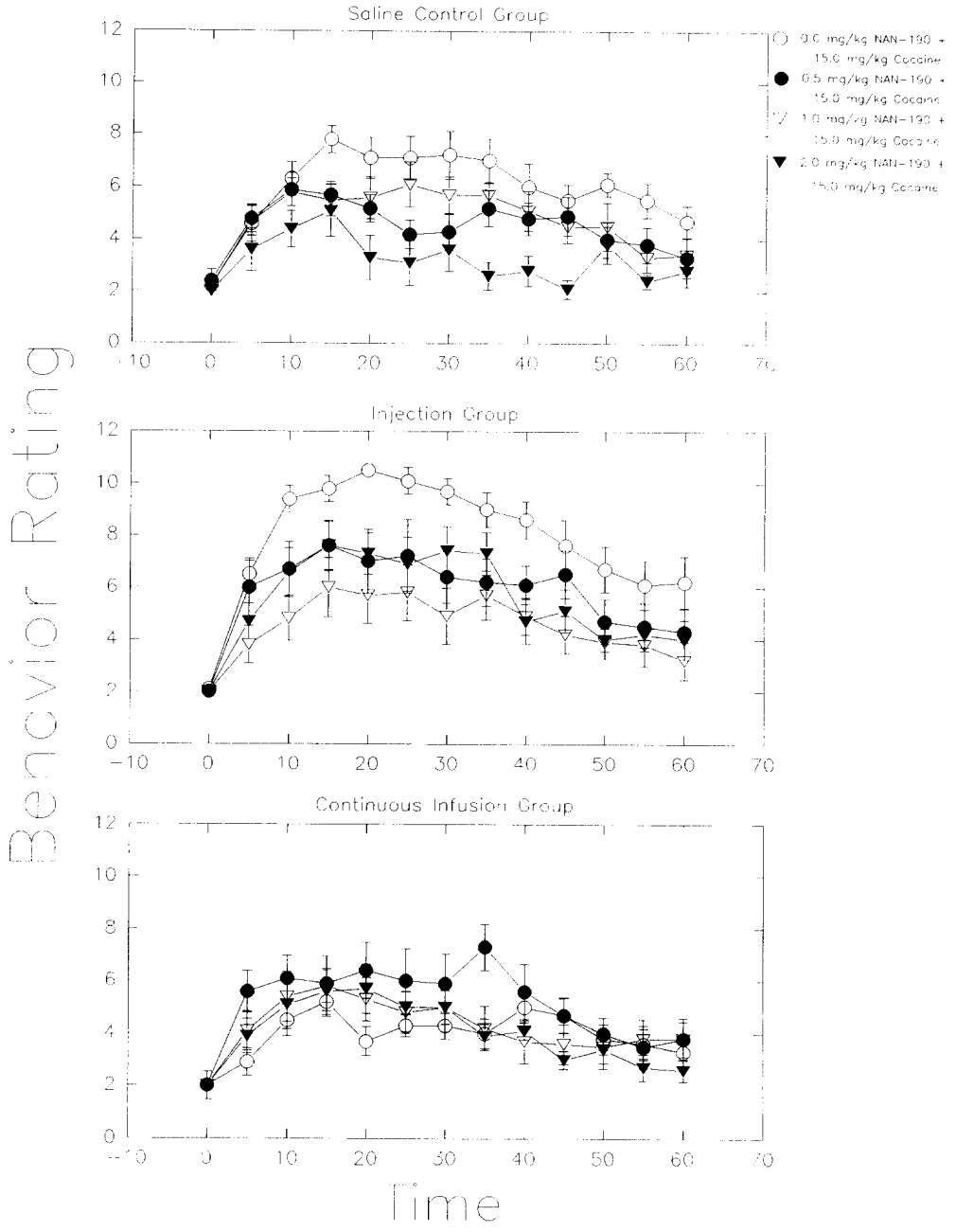

FIG. 3. Mean behavior rating for each combination of cocaine and NAN-190 separately for each pretreatment group. The bars represent 1 SE. (○), 0.0-mg/kg NAN-190 + 15-mg/kg cocaine dose; (●), 0.5-mg/kg NAN-190 + 15-mg/kg cocaine dose; (△), 1.0-mg/kg NAN-190 + 15-mg/kg cocaine dose; (▲), 2.0-mg/kg NAN-190 + 15-mg/kg cocaine dose.

pretreatment groups at 5–50 min. Mann–Whitney *U*-tests comparing the saline control and cocaine injection group indicated that there were significant differences at 5–40 min. Mann–Whitney *U*-tests comparing the saline control and continuous-infusion groups indicate significant differences at 15–35 and 50 min. Mann–Whitney *U*-tests comparing the injection and continuous-infusion groups indicate significant differences from 5–50 min.

Panel B presents the behavior ratings for subjects receiving a 0.5-mg/kg NAN-190 and 15-mg/kg cocaine combination dose. The results of Kruskall–Wallis ANOVAs by ranks did not indicate any significant differences at any time point.

Panel C presents the behavior ratings for subjects receiving a 1.0-mg/kg NAN-190 and 15-mg/kg cocaine combination dose. The results of Kruskall–Wallis ANOVAs by ranks did not indicate any significant differences at any time point.

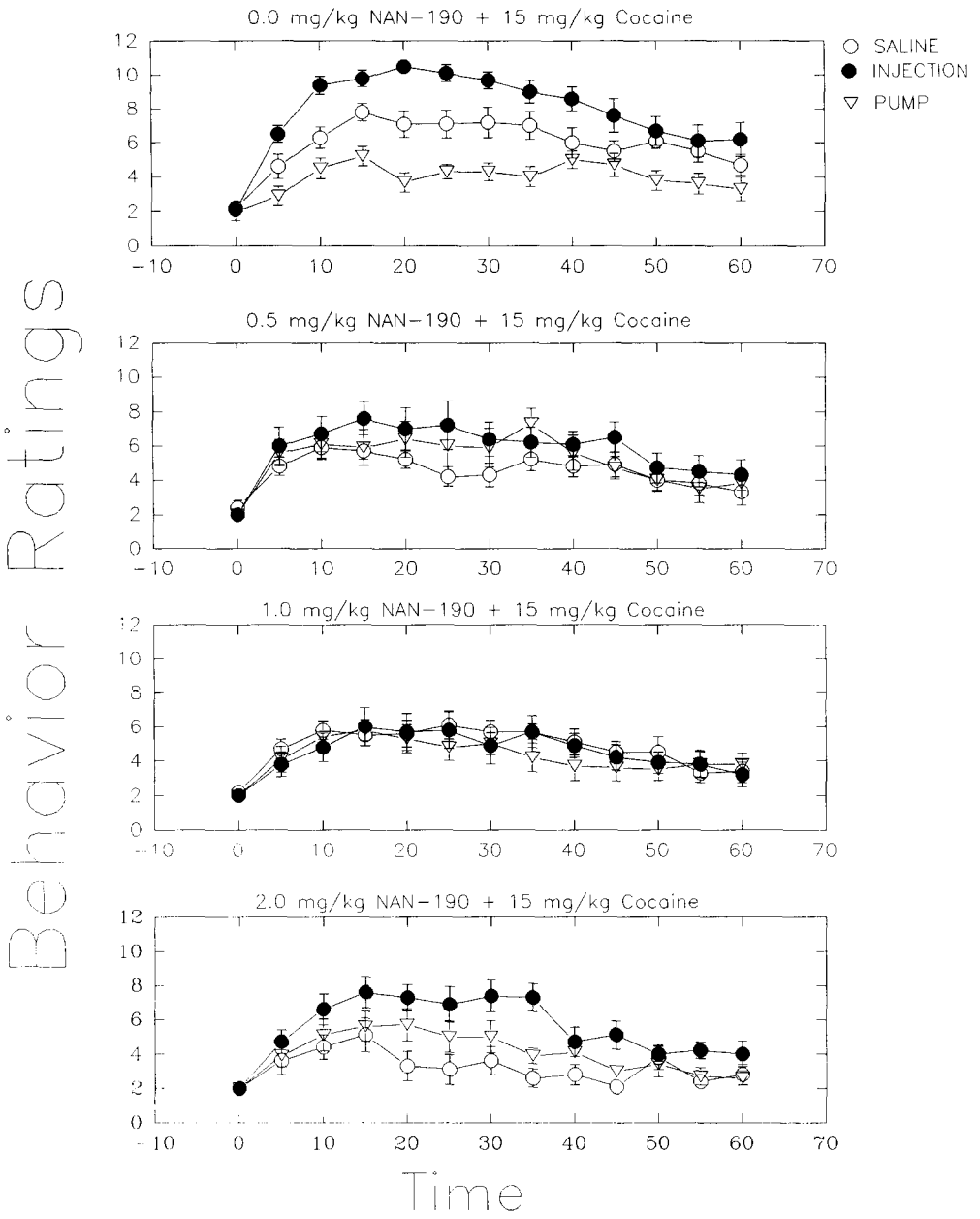

FIG. 4. Mean behavior rating for each group separately for each combination of cocaine and NAN-190. The bars represent 1 SE. (○), saline pretreatment rats; (●), cocaine injection pretreatment rats; (△), continuous-infusion pretreatment rats.

Panel D presents the behavior ratings for subjects receiving a 2.0-mg/kg NAN-190 and 15-mg/kg cocaine combination dose. The results of Kruskall-Wallis ANOVAs by ranks indicated significant differences between the pretreatment groups at 20-35, 45, and 55 min. Mann-Whitney *U*-tests comparing the different pretreatment groups indicated that the behavior ratings for the saline control group were significantly smaller than the behavior ratings for the injection group at 20-35, 45, and 55 min. The behavior ratings for the continuous-infusion group were significantly smaller than those for the injection group at 35, 45, and 55 min. The saline control and continuous-infusion groups were not significantly different at any time point.

Because of the differential effects of pretreatment on the response to a 15-mg/kg cocaine injection (i.e., sensitization or tolerance), changes in the sensitivity of 5-HT$_{1A}$ receptors were examined by determining the differences between this dose and the subsequent combinations of cocaine and NAN-190 separately for each pretreatment group. Figure 5 presents the difference scores between the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine dose and the other combinations of cocaine and NAN-190 for each pretreatment group separately for each

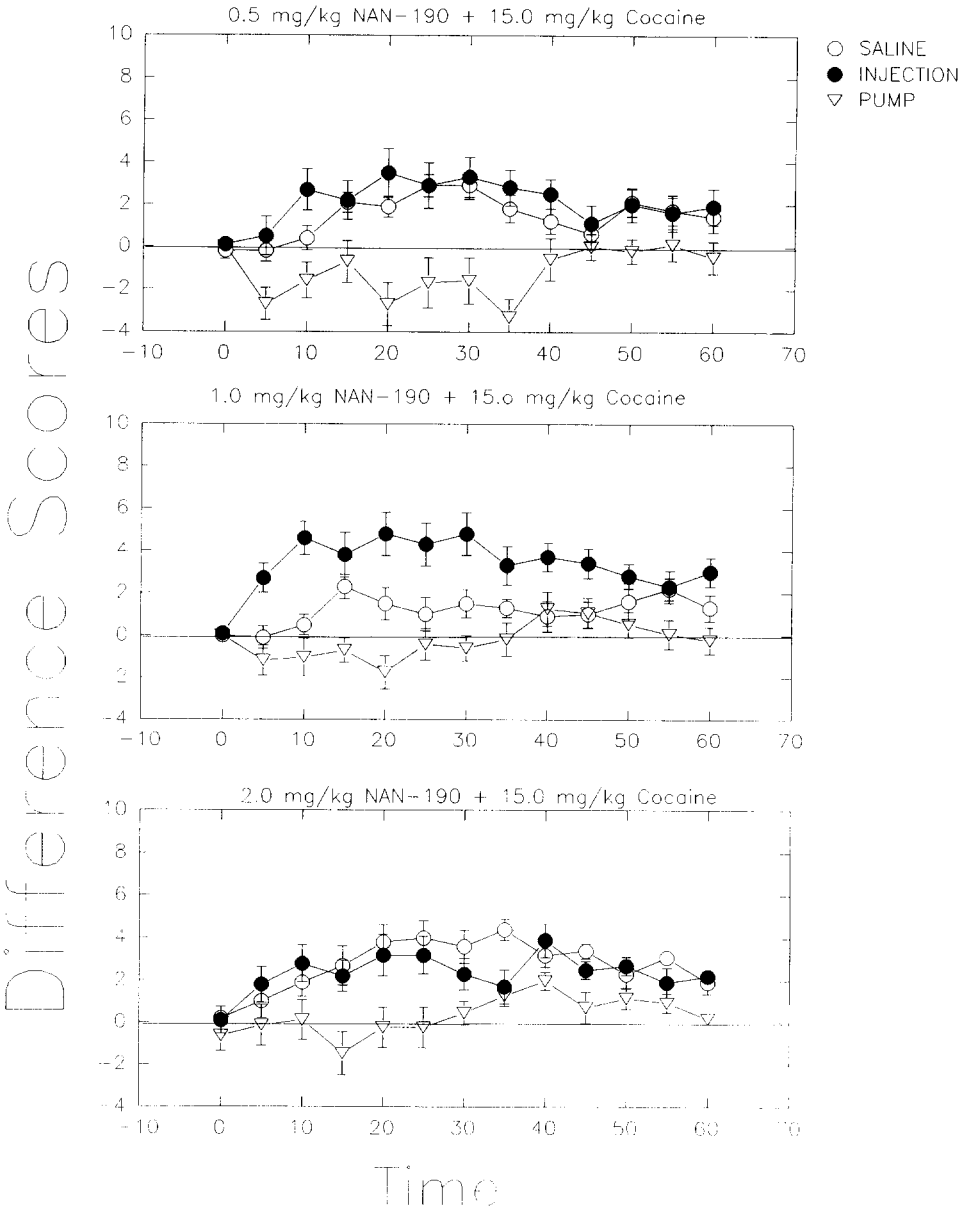

FIG. 5. Difference scores between 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine dose and the different combinations of cocaine and NAN-190 for each pretreatment group separately for each combination of cocaine and NAN-190. The bars represent 1 SE. (○), saline pretreatment rats; (●), cocaine injection pretreatment rats; (△), continuous-infusion pretreatment rats.

combination. In this figure, the larger the difference score the greater the effect of the particular dose of NAN-190 on cocaine-induced behavior.

Panel A presents the differences in behavior ratings between the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine and the 0.5-mg/kg NAN-190 plus 15-mg/kg cocaine combination doses, separately for each pretreatment group. The results of the Kruskall-Wallis ANOVAs by ranks indicated significant differences between the pretreatment groups at 0–35, 40, and 50 min. Mann-Whitney *U*-tests comparing the different pretreatment groups indicated that the difference scores for the saline control group were significantly smaller than the difference scores for the injection group at 0 and 10 min. Mann-Whitney *U*-tests comparing the saline control and continuous-infusion groups indicate significant differences at 0, 20–35, and 50 min. Mann-Whitney *U*-tests comparing the injection and continuous-infusion groups indicate significant differences at 0–10, 20–40, and 50 min.

Panel B presents the differences in behavior ratings between the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine and the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine combination doses separately for each pretreatment group. The results of Kruskall-Wallis ANOVAs by ranks indicated significant differences between the pretreatment groups at 0–40 and 60 min.

Mann–Whitney *U*-tests comparing the different pretreatment groups indicated that the difference scores for the saline control group were significantly smaller than the difference scores for the injection group at 0–10, 20–30, and 40–45 min. Mann–Whitney *U*-tests comparing the saline control and continuous-infusion groups indicate significant differences at 0, 15, and 20 min. Mann–Whitney *U*-tests comparing the injection and continuous-infusion groups indicate significant differences at 0–40 min.

Panel C presents the differences in behavior ratings between the 0.0-mg/kg NAN-190 plus 15-mg/kg cocaine and the 2.0-mg/kg NAN-190 plus 15-mg/kg cocaine combination doses separately for each pretreatment group. The results of Kruskall–Wallis ANOVAs by ranks indicated significant differences between the pretreatment groups at 0, 20–40, and 55 min. Mann–Whitney *U*-tests comparing the different pretreatment groups indicated that the difference scores for the saline control group were significantly smaller than the difference scores for the injection group at 0 and 35 min. Mann–Whitney *U*-tests comparing the saline control and continuous-infusion groups indicate significant differences at 0, 20–40, and 55 min. Mann–Whitney *U*-tests comparing the injection and continuous-infusion groups indicate significant differences at 0, 20, and 40 min.

DISCUSSION

The present results support and extend previous findings that indicate that the effects of chronic cocaine depend upon the route and temporal pattern of administration. Chronic, daily SC injections of cocaine produce sensitization to a subsequent cocaine challenge; this result is consistent with previous research using daily, intermittent injections of cocaine (22, 27,31). In contrast to these results, continuous infusion of an overall, equivalent daily dose of cocaine produces tolerance to a subsequent cocaine challenge. These results are consistent with the studies by Reith et al. (29) and King et al. (21). The present results also indicate that the 5-HT_{1A} antagonist NAN-190 can attenuate the locomotor-stimulating and stereotypy-inducing properties of cocaine injections. Hence, alterations in 5-HT functioning can modify the effects of cocaine.

The present experiments hypothesized the alterations in 5-HT_{1A} receptor sensitivity partially mediate the behaviors during withdrawal from chronic or intermittent cocaine administration. For example, we hypothesized that behavioral sensitization could result from 5-HT_{1A} supersensitivity. This supersensitivity would result in a substantial inhibition of 5-HT neuron firing at low cocaine doses. The decreased firing rate would result in a decreased 5-HT release in the mesolimbic DA systems. As a result, this DA system would be relatively disinhibited. Under such conditions, one would expect an enhanced behavioral response to dopamine-mediated behaviors (i.e., sensitization). Conversely, 5-HT_{1A} receptor subsensitivity could contribute to the development of behavioral tolerance to cocaine found with the continuous infusion of cocaine in an opposite manner (21). The present results are consistent with this hypothesis.

The present results indicate that the continuous-infusion group exhibited 5-HT_{1A} receptor subsensitivity. The saline control group (i.e., rats receiving chronic saline injections) demonstrated a dose-dependent decrease in cocaine-induced locomotor activity with concurrent administration of NAN-190. In contrast, continuous-infusion subjects did not demonstrate any effect of NAN-190 on cocaine-induced locomotor activity. In other words, there was no dose-dependent suppression of cocaine-induced locomotion with concurrent administration of NAN-190. In fact, there was a slight but nonsignificant increase in behavior during the NAN-190 plus 15-mg/kg cocaine conditions. Further, when the difference scores from the 15-mg/kg cocaine injection were determined they also failed to demonstrate any significant dose-dependent changes in behavior.

The results for the daily injection group are mixed regarding the issue of 5-HT_{1A} receptor supersensitivity. The results from the 0.5-mg/kg NAN-190 plus 15-mg/kg cocaine and the 1.0-mg/kg NAN-190 plus 15-mg/kg cocaine injections would seem to be indicative of receptor supersensitivity. In both cases, the sensitization typically found with the pretreatment regimen was eliminated. Further, analysis of the difference scores (i.e., Fig. 5) also indicate a relatively greater inhibition of cocaine-induced locomotion/stereotypy than that found in the saline control group. These results are consistent with electrophysiological research indicating that chronic, daily cocaine injections result in 5-HT_{1A} receptor supersensitivity (6,9).

In contrast to the results found with lower doses of NAN-190, the 2.0-mg/kg NAN-190 plus cocaine combination did not result in inhibition of the sensitization typically found with the pretreatment regimen. The behavior ratings of daily-injection subjects were significantly higher than subjects in the saline control group during this condition. Further, when this drug combination was considered there was no dose response to the NAN-190/cocaine combinations. The results from this drug combination would indicate receptor subsensitivity (i.e., tolerance or a biphasic effect) and not supersensitivity.

Although there is considerable evidence indicating the NAN-190 is a potent and selective postsynaptic 5-HT_{1A} receptor antagonist (16) there is also some evidence that NAN-190 acts as an agonist at presynaptic 5-HT_{1A} autoreceptors. Hjorth and Sharp (16), using microdialysis techniques, found that NAN-190 dose dependently decreased 5-HT release, although this effect was less than the inhibition produced by 8-OH-DPAT (17).

The results of the 2.0-mg/kg NAN-190 plus cocaine combination in the injection group may result from the mixed agonist/antagonist actions of NAN-190 acting on dorsal raphe 5-HT_{1A} autoreceptors. Inhibition of 5-HT release would have the effect of disinhibiting the mesolimbic DA system, resulting in a relative increase in locomotor and/or stereotypy scores. If the present results are due to the agonist actions of NAN-190 on presynaptic 5-HT autoreceptors, then these results are still consistent with the hypothesis that daily, intermittent injections of cocaine produce 5-HT_{1A} receptor supersensitivity because this effect was not exhibited by either the saline control or continuous-infusion groups. These results are consistent with the electrophysiological evidence of 5-HT_{1A} receptor supersensitivity. Future research should examine the issue of regional changes in receptor sensitivity.

In summary, the present results indicate that daily, intermittent SC injections produce sensitization to subsequent challenge doses of cocaine administered either 1 or 7 days after cessation of chronic treatment. In contrast to these results, continuous infusion of equivalent daily doses of cocaine results in tolerance to subsequent challenge doses of cocaine. Further, continuous infusion of cocaine results in 5-HT_{1A} receptor subsensitivity while daily, intermittent injections of cocaine probably produce 5-HT_{1A} receptor supersensitivity. Changes in 5-HT_{1A} receptor sensitivity may represent a partial mechanism for the development of sensitization and toler-

ance. Such changes in receptor sensitivity may also partially mediate the anergia and anhedonia experienced by human cocaine abusers during withdrawal (33). Lastly, the continuous-infusion paradigm may represent an alternative animal model of some aspects of human cocaine abuse.

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